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(54) Title: DERIVATIZED TRIS-CATECHOL CHELATING AGENTS

(57) Abstract

Bifunctional chelating agents are designed to sequester certain radioactive metals, such as gallium (III) isotopes, and to provide a means for covalently attaching these radionuclides to macromolecules, such as monoclonal antibodies. These chelating agents may be used in various therapeutic and diagnostic methods, such as in radioimaging and positron emission tomography.

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DERIVATIZED TRIS-CATECHOL CHELATING AGENTS

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to novel "bifunctional" chelating agents. More specifically, the present invention relates to bifunctional chelating agents which are designed to sequester certain radioactive metals and to provide a means for covalently attaching these radionuclides to a macromolecule, such as an antibody. The invention further relates to methods for preparing these compounds as well as methods of using these compounds in radioimmunoimaging, positron emission tomography and *in vivo* treatment. The present invention further relates to these compounds attached to antibodies.

Description of Related Art

Effective therapeutic methods for the treatment of cellular disorders such as cancer have been the object of intensive research. Conventional therapy employs surgery, radiation and chemotherapy. Each of these methods suffers a serious drawback in that it is not highly selective between healthy and cancerous cells. In order to be effective, these methods kill or remove large amounts of healthy tissue. Furthermore, chemotherapy adversely affects the immune system so that death or serious illness often arises from fungal, bacterial or viral infections.

The development of monoclonal antibodies has opened the possibility of selectively delivering therapeutic agents or diagnostic agents to specific target cells. Monoclonal antibodies are immunoglobulins of well-defined chemical structure. A characteristic feature of monoclonal antibodies is reproducibility of function and high specificity.

Diagnostic methods are adversely affected unless substantially all of the compound used for labeling is securely attached to the targeting agent. Any of the labelling compound that does not attach to the targeting agent can create an undesirable background. If radiometals are used, they can disseminate in the body and have the potential of doing damage.

U.S. Patent 4,454,106 relates to metal chelate conjugated monoclonal antibodies for diagnostic and therapeutic techniques. The chelating agent is derived from diethylenetriaminepentaacetic acid (DTPA), and the conjugate is substantially free of adventitiously bound metal. The chelate conjugated to the monoclonal antibody is a derivative of DTPA bonded to an organic functional group which serves to link the DTPA chelate to the monoclonal antibody.

Although the concept of attaching radionuclides to monoclonal antibodies with bifunctional chelates has been employed in some conventional methods, most conventional methods have utilized polyaminocarboxylates to coordinate the radionuclide. However, these chelators do not exhibit especial selectivity in their chelation of trivalent radiometals so trace divalent metal impurities may interfere in radiolabeling protocols. An increase in selectivity is desired and is of practical utility in that it would reduce the importance of the purity of radioactive metal ion solutions used for radiolabeling, thus lowering costs, and would increase stability of radiopharmaceutical preparations in vivo.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide novel chelating agents which overcome the above-noted problems and which sequester radioactive metals and

provide a means for covalently attaching the radionuclides to macromolecules.

It is another object of the present invention to provide a method for preparing bifunctional chelating agents.

It is a further object of the present invention to provide diagnostic and therapeutic techniques which employ these bifunctional chelating agents in the form of radiometal chelate conjugated monoclonal antibodies.

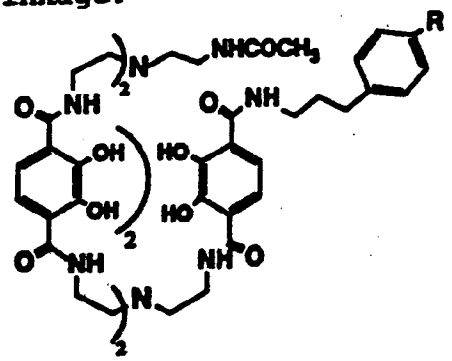
The foregoing objects and others are accomplished in accordance with the present invention, generally speaking, by providing bifunctional chelating agents having a tris-catechol structure and a method for preparing the same, wherein these agents are useful for sequestering radioactive metals (radionuclides) and for providing a means for covalently attaching these radionuclides to a macromolecule, such as an antibody. The present invention further encompasses therapeutic and diagnostic techniques which employ the bifunctional chelating agents in the form of radiometal chelate conjugated monoclonal antibodies.

Further scope of the applicability of the present invention will become apparent from the detailed description and drawings provided below. However, it should be understood that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides bifunctional chelating agents designed to sequester radioactive metals and to provide a means for covalently attaching these radionuclides to a macromolecule. When the macromolecule is a tumor-seeking monoclonal antibody, the radioactive conjugate can be used in patients for cancer diagnosis or therapy. The chelating agents of the present invention bind metals through three deprotonated 2,3-dihydroxyterephthalate moieties, known to have a much greater affinity for small, highly charged ions, such as Fe(III) and Ga(III), than for divalent ions, such as Ca(II) or Cu(II). In addition, two of the binding subunits are endocyclic within a 26 membered ring, a feature which should enhance the kinetic stability of the metal complex.

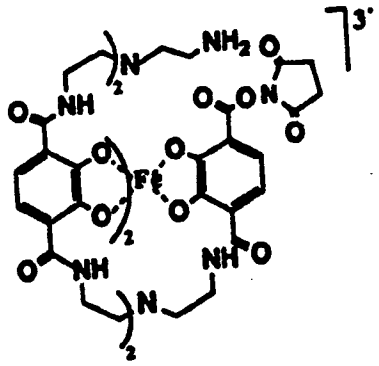
The bifunctional chelating agents of the present invention are represented by the formulas 1 and 1a shown below. Notable features of the chelating agents of the present invention include the 2,3-dihydroxyterephthalate binding subunits and the presence of a functionalized sidearm which allows the chelate to be covalently attached to a monoclonal antibody through, for example, a thiourea linkage.



1 R=NH_2

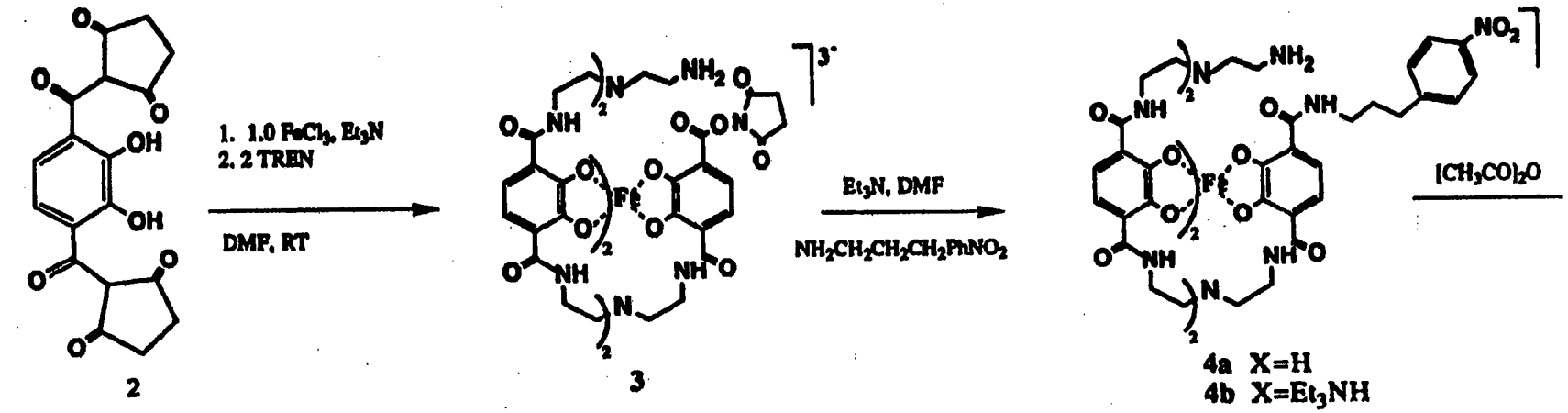
1a R=NCS

The above chelating agents are prepared in accordance with the present invention by derivatizing an intermediate 3 (below) in accordance with the synthetic procedures described for synthesizing macrobicyclic tris-catechol ligand in McMurry et al, J. Am. Chem. Soc., Vol. 109, pp. 3451-3453 (1987).



3

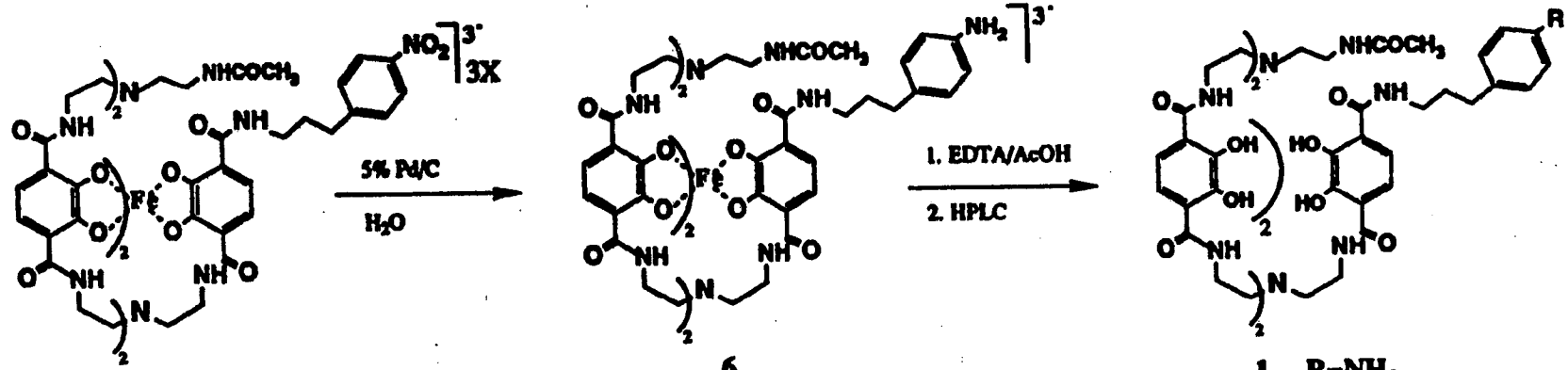
The synthetic scheme for the chelating agents of the present invention is summarized below.



2

3

4a X=H
4b X=Et_3NH



5a X=H
5b X=Et_3NH

6

1 R=NH_2

1a R=NCS

As shown in the scheme above, the disuccinimido-2,3-dibenzyloxyterephthalate **2** is reacted with two equivalents of the ligand tris(2-aminoethyl)amine (TREN) in the presence of iron (III) to give the metal complex **3**. This reactive intermediate may be reacted with 1-amino-2-(p-NO_2-Benzyl)ethane to give the derivatized complexes **4a,b**. The reactive alkylamine is then protected with acetic anhydride to give **5a, b** and then the aromatic amine reduced to provide the aniline metal complex derivative **6** that is subsequently demetalated to provide the amino ligand **1** which may be reacted with thiophosgene to give the isothiocyanate ligand **1a**. Both **1, 1a** are useful for linkage of the ligand to proteins, such as antibodies, by carbohydrate modification methods for **1** and by direct reaction with amino acid residues with **1a**.

The present invention employs metal chelate conjugated monoclonal antibodies for diagnostic and therapeutic techniques, particularly <u>in vivo</u>. The metal may be radioactive, exhibit fluorogenic properties, exhibit paramagnetic properties or the like.

Monoclonal antibodies are immunoglobulins of well-defined chemical structure, in contrast to polyclonal antibodies which are heterogeneous mixtures. Reproducibility of function cannot be controlled for either polyclonal or autologous antibodies, whereas unaltered function is characteristic to monoclonal antibodies. Experimental techniques for obtaining monoclonal antibodies have been extensively discussed. A useful text is <u>Monoclonal Antibodies</u> (R.H. Kennett, T.J. McKearn & K.B. Bechtol eds. 1980). See also Koprowski et al. U.S. Patent 4,196,265 which is incorporated herein by r ference. Any monoclonal

antibody which exhibits cell binding or antigen binding at the cell targeted for therapy or which is catabolized to inside the cell membrane can be employed. The selection and production of suitable monoclonal antibodies is within the skill of the art.

The antibodies are generally maintained in an aqueous solution that contains an ionic compound. A physiologic normal saline solution is very often employed and is widely available. Other ionic solutions, such as those containing sodium or potassium phosphate, sodium bicarbonate and the like, are known in the art and may also be employed.

The invention contemplates an in vivo therapeutic procedure in which radiometal chelate conjugated monoclonal antibodies are introduced into the body and allowed to concentrate in the target region. There are a variety of radiometal isotopes which form stable complexes with the chelating agents of the present invention and emit cytotoxic beta or positron particles, or Auger electrons. Useful beta or positron particle emitting isotopes include but are not limited to Sc-46, Sc-47, Sc-48, Ga-66, and Ga-68. The choice of radionuclide to be used depends on the purpose of the use, whether diagnosis of therapy, and is within the skill of the art. The therapeutic effect occurs when the conjugates are near or in contact with and bind to the targeted cells. Cell death, it is believed, is a direct or indirect result of the radiation event of the radiometal which is positioned in close proximity to the cell.

The benefits of this aspect of the invention are several. First, th high specificity of the conjugated monoclonal antibody minimizes th total radiati n dosage.

Only enough radiation for th target cells need be employed. In addition, radiometal chelates generally are cleared rapidly from the body should the conjugated antibody be disrupted. The isotope can be short-lived and the affinity constant by which the isotope that is retained in the chelate is very high resulting in a stably bound metal. Finally, since the amount of radiometal employed is minimized, the radiation hazard to persons preparing and administering the radiometal chelate conjugated antibody is also minimized.

Because of the properties of the radiometal chelate conjugated monoclonal antibody employed by the present invention, tissue damage or whole body dose during therapy are markedly reduced as compared to that from presently employed methods of radiation therapy such as isotope implants, external radiation therapy such as isotope implants, external radiation therapy, and immunoradiotherapy employing iodine-131 labeled polyclonal or autologous antibodies. Additionally, both biological and physical half-lives of the targeting radiobiological may now be controlled, minimizing whole body radiation effects. Since radiation is targeted specifically to cell types (e.g. neoplastic cells), a therapeutic dose is delivered specifically to malignant cells, either localized or metastasized. The ability of radiometal chelate conjugated monoclonal antibody to provide an effective dose or therapeutic radiation specifically to metastasized cells is also unique and singularly useful for cancer therapy.

In one of its particularly preferred aspects, the present invention employs the metal chelate conjugated monoclonal antibody containing a positron emitting radiometal to tr at cellular disorders. It is desirable

in most applications that the radiometal have a half-life of less than about four days and decay rapidly to a stable isotope once the alpha particle is emitted. The preferred isotopes employed in the present invention are Ga-66 and 68. Particularly preferred is Ga-66 with a half life of 9.4 hr.

In another embodiment, the present invention contemplates in vivo diagnostic procedure which comprises introducing a metal chelate conjugated monoclonal antibody into the body, allowing sufficient time for the conjugate to localize and identifying the degree and location of localization, if any. The present invention also contemplates in vitro analytical procedures employing a chelate conjugated monoclonal antibody. The conjugated antibody of the present invention is substantially free of adventitiously or weakly chelated metal.

A variety of isotopes useful for diagnostic purposes form stable complexes with the chelate of the present invention. Gamma or positron emitting isotopes are particularly useful for imaging target sites both in vivo and in vitro in radioimaging procedures. Examples of gamma or positron emitting isotopes include Tc-99m, Ga-67, Ga-68m or In-111. In the event that gamma camera images are desired, Tc-99m in In-111 are preferred. For positron emission tomography, Sc-43, Sc-44, Fe-52, Co-55 and Ga-66,68 may be employed. For fluorescence diagnostic techniques, lanthanides may be employed, in particular, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm and Yb. Paramagnetic diagnostic techniques would typically employ the stable iron isotopes such as Fe-54, Fe-56, Fe-57 and Fe-58. Qualitative and quantitative measurements can be made with instrum ntati n sensitive to each of

these forms of emission, or properties (optical or magnetic), available in the art.

The metal chelate conjugated antibodies of this invention can be administered in vivo in any suitable pharmaceutical carrier. As noted earlier, a physiologic normal saline solution can appropriately be employed. Often the carrier will include a minor amount of carrier protein such as human serum albumin to stabilize the antibody. The concentration of metal chelate conjugated antibodies within the solution will be a matter of choice. Levels of about 0.5 mg per ml are readily attainable but the concentrations may vary considerably depending upon the specifics of any given application. Appropriate concentrations of biologically active materials in a carrier are routinely determined in the art.

The effective dose of radiation or metal content to be utilized for any application will also depend upon the particulars of that application. In treating tumors, for example, the dose will depend, inter alia, upon tumor burden, accessibility and the like. Somewhat similarly, the use of metal chelate conjugated antibodies for diagnostic purposes will depend, inter alia, upon the sensing apparatus employed, the location of the site to be examined and the like. In the event that the patient has circulating antigen in addition to those located at the site, the circulating antigens can be removed prior to the treatment. Such removal of antigens can be removed prior to treatment. Such removal of antigens can be accomplished, for example, by the use of unlabeled antibodies, or by plasmapheresis in which the patient's serum is treated to remove antigens.

The present invention also contemplates the above-described chelating agents attached to macromolecules, such as antibodies. For example, the antibody anti-Tac is known to localize in adult t-cell leukemias. The antibody B72.3 localizes on the surface of colon cancer cells, and the antibody B-1 localizes in B-cell lymphomas.

The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

Template synthesis of 4a

Disuccinimido-2,3-dibenzyloxyterephthalate (4.0 g, 6.99 mmol) was dissolved in 200 ml EtOAc and hydrogenolyzed under 1 atm H_2 in the presence of 1 g 5% PD-C (2 hours). The resulting suspension of product and catalyst was filtered onto #42 paper using a Buchner funnel, and the filtrate was evaporated to recover the small amount of soluble product. Using vacuum filtration, the product disuccinimido-2,3-dihydroxyterephthalate was washed into a 250 ml RB flask with 200 ml DMF and placed under an argon atmosphere. Triethylamine (6 ml, ca. 43 mmol) and a DMF solution of $FeCl_3$(.378 g, 2.32 mmol in ca. 30 ml DMF) were added simultaneously. The resulting dark reddish brown solution was transferred to addition funnel A and DMF added to make the total volume 250 ml.

To addition funnel B was added 250 ml dry DMF and TREN (0.676 g, 4.64 mmol). The contents of addition funnels A and B were added to 800 ml DMF over 6 hours at room temperature and the reaction subsequently stirred 14 hours. High pressure liquid chromatography (HPLC) assay (conditions #2) showed essentially one peak, the mono-active ester 3, at 10.27 minutes. To this s luti n was

added 3-(para-nitrophenyl)propylamine hydrochloride (0.7 g, 3.2 mmol) and triethylamine (1 ml, ca, 7 mmol). The solution was stirred for 12 hours (HPLC retention time of product, 11.19 minutes), and the DMF evaporated to dryness. Aqueous ammonium acetate (0.01 M, 350 ml) was added and the pH adjusted to 9.7 with NH_4OH. The solution was stirred 12 hours and the insoluble materials removed by filtration. The pH of the filtrate was adjusted to 6.8 with glacial acetic acid and the volume diluted to 400 ml with 0.01 M $AcONH_4$. Purification was achieved by HPLC using a Waters Delta Prep and a Waters Delta Pak preparative C-18 reverse phase column (30 x 300 mm, 15 micro spherical packing, 100 A pore size) with a mobile phase of A = H_2O and B = MeOH (both 0.01 M $AcONH_4$). In a typical run, 10 ml of the above solution (D) was loaded and the products eluted with a 0-100% B (10%/min) gradient at 40 ml/min. The fraction eluting between 8.7 - 9.7 minutes was collected. The procedure was repeated until all crude material was purified. The aqueous solutions of product were evaporated to dryness and redissolved in ca. 125 ml H_2O. The solution was acidified to pH 3.05 with glacial acetic acid, resulting in a blackish precipitate (the neutral ferric complex), which was collected on a medium frit and washed with ca. 60 ml H_2O and dried to give 0.988g (1.03 mmol, 45%). A summary of the HPLC results is provided below in Table 1.

Table 1

HPLC Program

time	ml/min	%A	%B	%D	Curve
init	10.0	100	0		
0.1	10.0	0	0	100	11
0.9	10.0	0	0	100	11
0.91	40.0	100	0	0	11
1.91	40.0	100	0	0	6
2.91	40.0	100	0	0	6
12.91	40.0	0	100	0	6
16.00	40.0	0	100	0	6
19.00	40.0	100	0	0	6
22.00	40.0	100	0	0	6
22.90	10	100	0	0	6
23.00	start over				

IR (nujol, cm^{-1}) 3380(br), 3200, 1606(br), 1545, 1460, 1363, 1340, 1320, 1229, 1196(s), 730(m), 651(m)

Conversion to the triethylammonium salt 4b

Triethylamine (0.27 ml, ca. 1.9 mmol) was added to neutral ferric complex **4a** (0.36 g, .36 mmol) suspended in 10 ml MeOH. The resulting deep burgundy solution was stirred 15 minutes, then evaporated to dryness. The solid was redissolved in methanol (10 ml) and again evaporated to dryness. The solid was vacuum dried 48 hours to give 0.4 g (0.33 mmol).

Elemental Analysis: Calc. for $[FeLH]2-[(Et_3NH+)_2]-2H_2O$ $FeC_{57}H_{72}N_{18}O_{14}$: C,54.76; H,6.85; N,13.44; Fe,4.47. Found C,54.71; H,7.02; N,13.47; Fe,4.21.

Synthesis of the acetamide 5a

Neutral ferric complex **4a** (0.497 g, 0.49 mmol) was dissolved in 8 ml anhydrous DMF under arg n by the

addition of Et_3N (.41 ml, ca. .3g, 2.9 mmol, 6 equiv.) was added and the reaction stirred 75 minutes. HPLC (conditions #2, Gilson) show no starting material (RT 11.07 min) and a single product (RT 10.37 min). The DMF was evaporated to give an oil, to which 10 ml H_2O was added. Glacial acetic acid was added to precipitate the ferric complex, which was collected on a fine glass frit, washed with water and dried to give 0.45 g (0.43 mmol, 87%).

Preparation of Et_3NH salt of acetamide **5b**

Neutral complex **5a** (0.15 g, .14 mmol) was suspended in 5 ml CH_3OH and stirred while triethylamine (0.12 ml, 0.086 g, .85 mmol) was added. The resulting burgundy solution was evaporated to dryness, redissolved in 20 ml methanol and again evaporated to dryness. The solid was taken up in ca. 1 ml methanol, precipitated by the addition of diethyl ether, and collected on a fine glass frit. The solid was vacuum dried to give 0.19 g (.14 mmol, 95%) of the triethylammonium salt **5b**.

Elemental Analysis: Calc for $[Fe(C_{47}H_5ON_{10}O_{15}]_3[C_6H_{16}N+]_3$ $3H_2O$:C,57.51; H,7.28; N,13.41; Fe,4.11. Found: C,55.31; H,7.43; N,12.90; Fe,3.96.

Reduction to aniline and demetallation to give **1**

Neutral ferric complex **5a** (0.4 g, .38 mmol) was suspended in 10 ml H_2O and solubilized by the addition of NaOH (1.2 ml 1M NaOH, 1.1 mmol). The pH of the solution was adjusted to 7.4, and was then transferred via syringe to a 50 ml 3 neck flask containing 250 mg 10% Pd/C (saturated with H_2). Hydrogenation at atmospheric pressure was complete in 7-8 hours, as evidenced by the analytical HPLC (conditions #2) which showed the complete c nversion of starting material (RT 10.4) to a single

product (RT 9.6). The catalyst was removed by filtration and washed with water (10 ml) (Buchner). To the filtrate was added $Na_2EDTA\ 2H_2O$ (1.41 g, 3.8 mmol, 10 eq) and glacial acetic acid (12 ml). The solution was stirred 2 hours at room temperature, then left to stand at room temperature 12 hours. The precipitate was filtered and washed with 15 ml 0.1 M AcOH. The filtrate was diluted to 100 ml with 0.1 M AcOH and purified by HPLC using a Gilson autoprep system and conditions #3. The fraction between 10.5 and 12 minutes was collected and evaporated to dryness. The brown solid (ca. 300 mg) was taken up in 140 ml boiling absolute ethanol and cooled to room temperature. Et_2O (125 ml) was added and the solution cooled to 4C. The resulting solid was collected, washed with Eto and dried to give 0.19 g (0.2 mmol, 52%) of the neutral tris(catecholate) ligand.

^1H NMR (D_2), pD=9.5): 7.07(d,2H,J=7.9Hz),
6.925(d,1H,J=8.5Hz)
6.725(d,2H,J=8.1Hz)
6.651(br.s.,4H)
6.575(d,2H,J=8.6Hz)
3.398(br.s.,6H), 3.291(br.s.,6H)
3.093(br.t.,2H)
2.731(br.s.,6H)
2.581(br.s.,6H)
2.449(br.t.,2H), 1.809(m,2H), 1.598(s,3H)

Synthesis of 1a

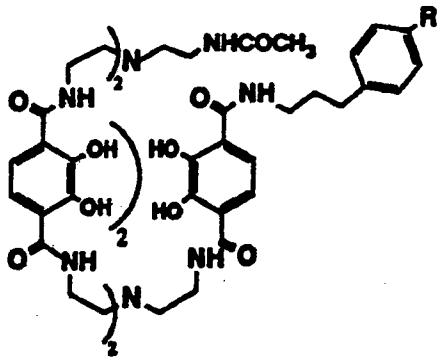
Compound 1 (27.8 mg, 26.1 μmol) was dissolved in 0.100 M NaOH (.813 mL). To this golden solution was added Cl_2CS (192 μL of a 0.17M $CHCl_3$ solution). The reaction was stirred vigorously for 3 minutes, during which time a greenish gray precipitate formed. The precipitate was concentrated by centrifugation, the

supernat nt removed, and the solid washed with distilled wat r. After vacuum drying, 25 mg of product was obtained. IR (nujol) 3300 (m), 2080(s).

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

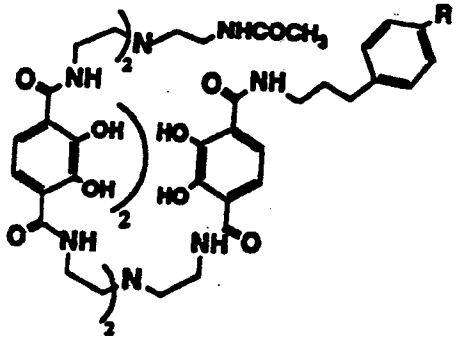
WHAT IS CLAIMED:

1. A compound of the formula:



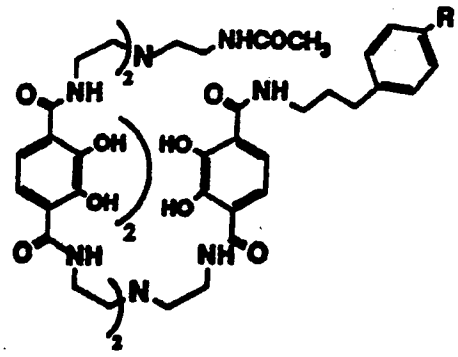
wherein R is $-NH_2$ or -NCS.

2. A compound of the formula:



wherein R is $-NH_2$ or -NCS, said compound being complexed with a radiometal isotope.

3. A pharmaceutical composition comprising a compound of the formula:



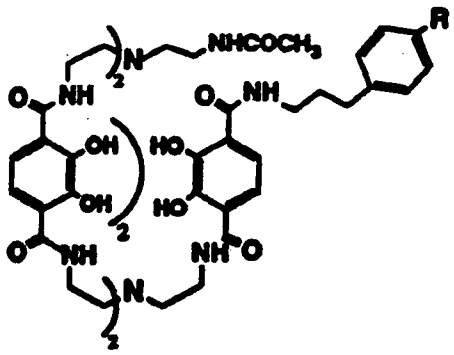
wh rein R is $-NH_2$ r -NCS, said compound being in the form of a radiometal chelate conjugated macromolecule or antibody; and a pharmac utically acceptable excipient.

4. Use of a solution of the radiometal chelate conjugated antibodies of claim 3 specific for a target cell for an in vivo diagnostic method for the treatment of cellular disorders wherein said solution is introduced into body fluid.

5. Use of a solution of the radiometal chelate conjugated antibodies of claim 3 for an in vitro diagnostic method which comprises introducing into a test medium said solution and quantifying the specifically bound portion of said conjugate.

6. Use according to claim 5 wherein quantifying is conducted by using radioimmunoimaging or positron emission tomography.

7. A method for preparing final product compounds of the formula:



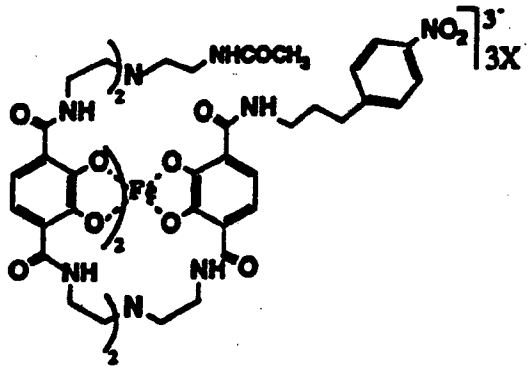
wherein R is $-NH_2$ or -NCS,

which method comprises the steps of:

reacting disuccinomido-2,3-dibenzyloxyterephthalate with tris(2-aminoethyl)amine in the presence of iron (III) to form an intermediate m tal c mplex;

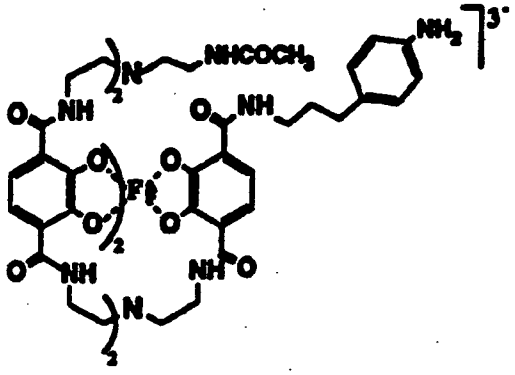
reacting said intermediate metal complex with 1-amino-2-(p-NO_2-Benzyl)ethane t form derivatized intermediate complexes;

reacting said derivatized intermediate complexes with acetic anhydride to form aromatic amine compounds of the following formula:



wherein X is H or Et_3NH;

reducing said aromatic amine compounds to form an aniline metal complex derivative of the formula:



; and

demetalating said aniline metal compl x derivative to f rm said final product compound.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/09153

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

INT CL.(5): A61K 49/02 C07D 245/00 G01N 23/00
U.S. CL.: 424/1.1 546/460 436/57

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	424/1.1 540/460 436/57,804 534/10,14

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	Journal of American Chemical Society, Volume 109, No. 11, issued November 1987 (USA), T.J. MCMURRAY ET. AL., 'Template and Stepwise Syntheses of a Macrobicyclic Catechoylamide Ferric Ion Sequestering Agent,' see pages 3451-3453, especially page 3452.	7
A	US, A, 4,732,974 (NICHOLOTTI ET. AL.) 22 MARCH 1988	1,7

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

28 FEBRUARY 1992

Date of Mailing of this International Search Report

12 MAR 1992

International Searching Authority

IPEA/US

Signature of Authorized Officer

JOHN S. MAPLES

FURTHER INF RMATION CONTINUED FROM THE SEC ND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . because they relate to subject matter [2] not required to be searched by this Authority, namely:

2. ☐ Claim numbers ... , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [3], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

I. Claims 1 and 7, drawn to a heterocyclic compound and method of making, classified in Class 540, subclass 460.

II. Claim 2, drawn to a radiolabeled complex, classified in Class 424, subclass 1.1.

III. Claims 3-4, drawn to a composition and method of use, classified in (Con't next

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	Class 424, subclass 1.1. IV. Claims 5-6, drawn to a method of in vitro use, classified in Class 436, subclass 57.	